

AMENDMENT UNDER 37 C.F.R. § 1.121

In response to the outstanding Office Action dated December 19, 2000, Applicants submit the following amendments and remarks. Please amend the subject application as follows:

In the Claims:

Cancel claims 19-34 and add Claims 35-60.

35. (new) A stable cell line of mammalian origin transformed with a vector comprising a DNA sequence encoding a protein of the formula:

His-Xaa<sup>1</sup>-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Xaa<sup>2</sup>-Gly-Gln-Ala-Ala-Xaa<sup>3</sup>-Xaa<sup>4</sup>-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa<sup>5</sup>

wherein

Xaa<sup>1</sup> is Ala, Gly, Val, Thr, or Ile;

Xaa<sup>2</sup> is Glu, Gln, Ala, Thr, Ser, or Gly;

Xaa<sup>3</sup> is Lys, or Arg;

Xaa<sup>4</sup> is Glu, Gln, Ala, Thr, Ser, or Gly; and,

Xaa<sup>5</sup> is Gly-OH or is absent, and

wherein the cells are immunologically masked.

36. (new) The cell line of Claim 35 wherein the cells are immunologically masked with a method selected from a group consisting of the following:
- a) coating the cells with F(ab')<sub>2</sub> fragments specific for HLA class I antigens or
  - b) encapsulating the cells in semi-permeable membranes.
37. (new) The cell line of Claim 35 wherein Xaa<sup>1</sup> is Ala or Val; Xaa<sup>2</sup> is Glu; Xaa<sup>3</sup> is Lys or Arg; Xaa<sup>4</sup> is Glu; and Xaa<sup>5</sup> is Gly-OH or is absent.
38. (new) The cell line of Claim 37 wherein Xaa<sup>1</sup> is Ala,

Xaa<sup>3</sup> is Lys; and Xaa<sup>5</sup> is Gly-OH.

39. (new) The cell line of Claim 37 wherein Xaa<sup>1</sup> is Val; Xaa<sup>3</sup> is Lys; and Xaa<sup>5</sup> is Gly-OH.
40. (new) The cell line of Claim 35 wherein the vector further comprises a viral promoter controlling expression of said DNA sequence.
41. (new) The cell line of Claim 35 wherein the vector further comprises a metallothionein promoter controlling expression of said DNA sequence.
42. (new) The cell line of Claim 35 wherein the DNA sequence is:  
5' - CAT GCT GAA GGG ACC TTT ACC AGT GAT GTA AGT TCT  
TAT TTG GAA GGC CAA GCT GCC AAG GAA TTC ATT GCT TGG  
CTG GTG AAA GGC CGA GGA - 3'.
43. (new) The cell line of Claim 35 wherein the DNA sequence is:  
5' CAT GTT GAA GGG ACC TTT ACC AGT GAT GTA AGT TCT  
TAT TTG GAA GGC CAA GCT GCC AAG GAA TTC ATT GCT TGG  
CTG GTG AAA GGC CGA GGA - 3'.
44. (new) The cell line of Claim 35 which is an immortalized cell line.
45. (new) The cell line of Claim 44 which is of human embryonal kidney cell origin.
46. (new) The cell line of Claim 35 which is transformed with a vector selected from the group consisting of:  
a) pGT-h+tLB+GLP-1;  
b) pGT-h+tLB+Val8GLP-1; and  
c) pMT-h+tLB+Val8GLP-1.

47. (new) The cell line of Claim 46 wherein the vector is pGT-h+tLP+GLP-1.
48. (new) The cell line of Claim 46 wherein the vector is pGT-h+tLB+Val8GLP-1.
49. (new) A method of producing an immunologically masked, transformed, stable cell line of mammalian origin which expresses a protein of the formula:

His-Xaa<sup>1</sup>-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Xaa<sup>2</sup>-Gly-Gln-Ala-Ala-Xaa<sup>3</sup>-Xaa<sup>4</sup>-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa<sup>5</sup>

wherein

Xaa<sup>1</sup> is Ala, Gly, Val, Thr, or Ile;

Xaa<sup>2</sup> is Glu, Gln, Ala, Thr, Ser, or Gly;

Xaa<sup>3</sup> is Lys, or Arg;

Xaa<sup>4</sup> is Glu, Gln, Ala, Thr, Ser, or Gly; and,

Xaa<sup>5</sup> is Gly-OH or is absent

comprising the steps of:

- a) providing a population of cells from a mammalian cell line;
- b) transfecting the cells of (a) with a vector comprising a DNA sequence encoding a protein of the formula:

His-Xaa<sup>1</sup>-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Xaa<sup>2</sup>-Gly-Gln-Ala-Ala-Xaa<sup>3</sup>-Xaa<sup>4</sup>-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa<sup>5</sup>

wherein

Xaa<sup>1</sup> is Ala, Gly, Val, Thr, or Ile;

Xaa<sup>2</sup> is Glu, Gln, Ala, Thr, Ser, or Gly;

Xaa<sup>3</sup> is Lys, or Arg;

Xaa<sup>4</sup> is Glu, Gln, Ala, Thr, Ser, or Gly; and,

Xaa<sup>5</sup> is Gly-OH or is absent,

- c) culturing the transfected cells of (b), under conditions that selectively propagate cells which

express the protein of (b), and  
d) immunologically masking the cells of (c).

50. (new) The method of Claim 49 wherein the cells are immunologically masked using a method selected from the group consisting of the following:  
a) coating the cells with F(ab')<sub>2</sub> fragments specific for HLA class I antigens; or  
b) encapsulating the cells in a semi-permeable membrane.
51. (new) The method of Claim 49 wherein Xaa<sup>1</sup> is Ala or Val; Xaa<sup>2</sup> is Glu; Xaa<sup>3</sup> is Lys or Arg; Xaa<sup>4</sup> is Glu; and Xaa<sup>5</sup> is Gly-OH or is absent.
52. (new) The method of Claim 49 wherein Xaa<sup>1</sup> is Ala, Xaa<sup>3</sup> is Lys; and Xaa<sup>5</sup> is Gly-OH.
53. (new) The method of Claim 49 wherein Xaa<sup>1</sup> is Val; Xaa<sup>3</sup> is Lys; and Xaa<sup>5</sup> is Gly-OH.
54. (new) The method of Claim 49 wherein the vector further comprises a viral promoter controlling expression of said DNA sequence.
55. (new) The method of Claim 49 wherein the vector further comprises a metallothionein promoter controlling expression of said DNA sequence.
56. (new) The method of Claim 49 wherein the vector is selected from the group consisting of:  
a) pGT-h+tLB+GLP-1;  
b) pGT-h+tLB+Val8GLP-1; and  
c) pMT-h+tLB+Val8GLP-1.
57. (new) The method of Claim 56 wherein the vector is

pGT-h+tLP+GLP-1.

58. (new) The method of Claim 56 wherein the vector is pGT-h+tLB+Val8GLP-1.
59. (new) A method of inducing insulin expression in a mammal in need thereof comprising,  
a) preparing an expression vector capable of expressing a protein with the sequence of SEQ ID NO: 1,  
b) introducing the expression vector into the mammal whereby the expression vector, when incorporated into a cell, produces a protein of SEQ ID NO: 1.
60. (new) A method of inducing insulin expression in a mammal in need thereof comprising,  
a) preparing, using the method of Claim 49, an immunologically masked transformed stable cell line that expresses a protein with the sequence shown in SEQ ID NO: 1  
b) introducing cells of the stable cell line of (a) into the mammal whereby the protein with the sequence shown in SEQ ID NO: 1 is expressed in the introduced cells in the mammal.

**REMARKS**

Reconsideration of the application and entry of the amendments is requested. The amended claims 35-60 are fully supported by the present specification and no new matter has been introduced. Applicants respectfully request entry of these amended claims. Amended Claims 35 through 48 claim an immunologically masked, stable cell line of mammalian origin expressing a GLP-1 protein with the sequence shown in SEQ ID NO: 1. Support for these claims is found throughout the specification and specifically on pages 10-11 and 22. Amended Claims 49 through 58 claim a method of producing said cell